PATENT SPECIFICATION (11) 1 402 751

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(21) Application No. 8842/72 (22) Filed 10 Jul 1972

(31) Convention Application No. 2137617 (32) Filed 28 Jul 1971 in

(33) Germany (DT)

(44) Complete Specification Published 13 Aug 1975

(51) INT. CL.[2] C07C 143/14
C25B 3/00

(52) Index at acceptance C2C 20Y 290 292 29Y 304 30Y
321 322 323 32Y 394 39Y
605 640 797 799 79Y RK
C7B 10B

(54) "AMPHOLYTIC MATERIALS."

I, NIKOLAUS GRUBHOFER, of Gustav-Kirchhoffstrasse 12, D-69, Heidelberg, Germany of German Nationality, do hereby delcare the invention, for which I pray that a patent may be granted to me, and the method by which it is to be performed to be particularly described in and by the following statement:-

This invention is concerned with improvements in and relating to ampholytic materials and the preparation thereof. As is well known, ampholytes are compounds containing both acidic and basic groups and this invention is particularly concerned with ampholytic materials comprising a mixture of different ampholytes having varying ratios of acidic to basic groups.

In the absence of any other electrolyte, an aqueous solution of an ampholyte has a definite pH value, namely that corresponding to the iso-electric point of the ampholyte. Depending on whether it contains a preponderant of basic or acidic groups an ampholyte migrates to the anode or cathode when an electric field is applied. In the case of a mixture of different ampholytes, the ampholytes will initially neutralise each other but when an electrical field is applied to a solution thereof the ampholytes will travel at different rates and in different directions depending on their iso-electric point. If mixing of the aqueous phase is prevented by stabilization in membranes, a pH gradient will be caused in such an ampholyte mixture after the application of an electrical field. The pH gradient may range from a strongly acidic pH close to the anode to a strongly alkaline pH close to the cathode. The generation of such a pH gradient may be employed for very precise electrophoretic separations of high molecular weight ampholytes, for example albumen substances, in order to analyse or purify them. This separation process may be termed "focussing electrophoresis".

Ampholytes which have been used for focussing electrophoresis contain primary secondary and tertiary amino groups as the basic groups, and carboxyl groups as the acid groups.

However, such ampholytes suffer from a number of disadvantages, as follows. Firstly, the use of carboxylic acid groups as acidic groups does not allow the iso-electric point to be reduced below about pH4.5, thus restricting the acid limit of the pH gradient. Secondly, it is difficult to obtain higher iso-electric points than pH 10 using primary, secondary or tertiary amino groups as basic groups. Finally, the use of carboxyl groups as acidic groups may give rise to problems in the treatment of biological material since carboxyl groups may form insoluble or complex compounds with multivalent metal cations, in particular with calcium and magnesium ions, but also and to an increased extent, with copper, zinc or like ions, which may be encountered in biological research.

It has now been found, in accordance with the present invention, that ampholyte materials may be prepared in which sulphonic acid groups, rather than carboxylic acid groups, serve as the acidic groups.

Accordingly, one mebodiment of the present invention provides an ampholytic material comprising a mixture of ampholytes which are polyamines containing at least four primary, secondary, tertiary or quaternary amino groups, as basic groups, and at least one sulphonic acid or sulphonic acid ester group as acidic group.

The ampholytic material in accordance with the invention may be prepared by reacting a polyamine containing at least four primary, secondary or tertiary amino groups with a molar excess of an alkylating agent which introduces an alkyl group bearing a sulphonicacid or sulphonic acid ester group into the primary or secondary amino groups of the polyamine. Examples of suitable alkylating agents include alkane sultones, e.g. propane sultone, haloalkyl sulphonic acids, especially bromoalkyl sulphonic acids such as bromoethanesulphonic acid, and

unsaturated aliphatic sulphonic acids such as vinyl sulphonic acid. The polyamine is preferably an aliphatic polyamine and may be, for example, a polyalkylene polyamine containing at least four amino groups such as pentaethylene hexamine. The reaction between the polyamine and the alkylating agent may suitably be carried out in the presence of a catalyst for example barium hydroxide and, if desired, in the presence of an antioxidant for example barium sulphate.

It has also been found, in accordance with another embodiment of the invention, that the basicity of the amino groups may be increased by reaction with a suitable alkylating agent, without adversely affecting the preparation of the ampholyte mixture.

Thus, before reaction of the polyamine with the alkylating agent the polyamine may be partially quaternised with a neutral alkylating agent, e.g. an alkyl halide such as methyl iodide, or dimethyl sulphate, and the acid reaction product formed is suitably removed by treatment with a cation exchanger. Where the alkylating agent is an alkyl halide quaternisation is suitably carried out in metharolic solution and when the alkylating agent is dimethyl sulphate quatanisation is suitably carried out in aqueous solution.

The reaction products formed by reaction of the polyamine and alkylating agent are mixed together to form a mixture contain many ampholytes having iso-electric points situated at varyingly close intervals. The wide variation in reaction conditions for the preparation according to the invention of such ampholytes is a special advantage of the method.

The ampholyte mixtures obtained in accordance with the invention may be analysed by electrophoretically separating the mixture on a polyacrylamide or dextran-gel carrier, and measuring the pH value at different points of the range of separation. The separation of the ampholyte mixture to obtain separate fractions having a particular narrower range of iso-electric points, may be carried out using known electrophoretic systems.

The ampholyte materials produced in accordance with the invention may be used, for example, in the separation and analysis of high molecular weight ampholytes, e.g. protein and mucopolysaccharides, using electrophoretic focussing.

In order that the invention may be well understood the following Examples which are given by way of illustration only. In the Examples reference will be made to the accompanying drawings which are graphical representations of the pH value of electrophetically separated compositions produced in accordance with the invention.

EXAMPLE 1

(a) Quaternarisation of polyamine:

2.32g (0.01 mole) of pentaethylenehexamine is dissolved in 20 cc of methanol, mixed with 5.04g (0.04 mole) of dimethyl sulphate, and boiled under reflux for 4 hours. 50 cc of water is added to the reaction mixture which is then reduced to half its volume under vacuum. The methylsulphuric acid produced is extracted in a column using an acidic ion exchanger (Dowex 1 x 8).

(b) Reaction of a polyamine with propane sultone 2.32g (0.01 mole) of pentaethylenehexamine in 30 cc of a 10% solution of $Ba(OH)_2 . 8H_2O$ in water, or the equivalent quantity of the mixture obtained in (a) above, is mixed with 3.05g (0.025 mole) of propane sultone dissolved in 25 cc of acetone, and boiled under reflux for 4 hours. The barium is removed by the addition of dilute sulphuric acid.

EXAMPLE 2

Reaction of polyamine with bromoethanesulphonic acid:

2.32g (0.01 mole) of pentaethylenehexamine dissolved in 30 cc of water, or the equivalent quantity of the mixture obtained in Example 1 (a), is mixed with, 4.70g (0.025 mole) of bromoethanesulphonic acid and boiled under refulux for 4 hours.

EXAMPLE 3

Reaction of polyamine with vinylsulphonic acid:

2.32g (0.01 mole) of pentaethylenehexamine dissolved in 30 cc of water or the equivalent quantity of the mixture obtained in Example 1(a), is mixed with, 3.25g of sodium vinyl sulphonate and boiled under reflux conditions for 4 hours.

The pH gradients of the materials obtained in the above examples were determined as follows:

The mixture produced in the Example was freed from acetone by vacuum distillation, made up to a volume of 20 cc with water to give a solution II which was used in the production of a polyacrylamide gel from the following indgredients:

solution II	2.5 cc
40% acrylamide	3.75 "
water	5.75 "
0.1% persulphate solution	12.00 "
0.8% tetraethylene tetramine	0.03 "

The mixture is poured into a glass dish (80 mm x 100 mm x 3 mm) and exposed to electrophoresis at 100 volts after polymerisation. The gradient forming action is completed after approximately 3 hours: the electrophoresis is interrupted and the gel is removed from the dish and cut into twelve strips each having a width of 6 mm, extending along the gradient. The separate strips are each triturated with 4 cc of water and centrifuged. The pH value of each triturate is then measured by electrometry.

The results are shown in the accompanying drawings.

What I claim is:—

1. An ampholytic material comprising a mixture of ampholytes which are polyamines containing at least four primary, secondary, tertiary or quaternary amine groups, as basic groups and at least one sulphonic acid or sulphonic acid ester group, as acidic group.

2. An ampholytic material as claimed in claim 1 substantially as hereinbefore described with reference to the Examples.

3. A process for the preparation of an ampholytic material as claimed in claim 1 which comprises reacting a polyamine containing at least four primary, secondary or tertiary amino groups with a molar excess of an alkylating agent which introduces an alkyl group bearing a sulphonic acid or sulphonic acid ester group into the primary or secondary amino groups of the polyamine.

4. A process as claimed in claim 3, in which the alkylating agent is an alkane sultone, a haloalkyl sulphonic acid or an unstaturated aliphatic sulphonic acid.

5. A process as claimed in claim 4 in which the alkylating agent is propane sultone, a bromoalkyl sulphonic acid or vinyl sulphonic acid.

6. A process as claimed in any one of claims 3–5, in which the polyamine is an aliphatic polyamine.

7. A process as claimed in claim 6 in which the aliphatic polyamine is a polyalkylene polyamine containing at least four amino groups.

8. A process as claimed in any one of claims 3–7 in which the reaction is carried out in the presence of a catalyst.

9. A process as claimed in any one of claims 3–8 in which the reaction is carried out in the presence of an antioxidant.

10. A process as claimed in any one of claims 3–9 in which the polyamine is first quaternised with a neutral alkylating agent and the acid reaction product formed is removed by treatment with a cation exchanger.

11. A process as claimed in claim 10, in which the neutral alkylating agent is an alkyl halide and the quaternisation is carried out in methanolic solution.

12. A process as claimed in claim 10 in which the neutral alkylating agent is dimethyl sulphate and the quaternisation is carried out in aqueous solution.

13. A process as claimed in claim 3 substantially as hereinbefore described with reference to the Examples.

14. Ampholytic materials when obtained by a process as claimed in any one of claims 3–13.

15. A process for the separation or analysis of high molecular weight ampholytes by electrophoretic focussing in which there is used an ampholyte material as claimed in any one of claims 1, 2 and 14.

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Printed for Her Majesty's Stationery Office, by Croydon Printing Company Limited, Croydon, Surrey, 1975.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.

1402751 COMPLETE SPECIFICATION
2 SHEETS This drawing is a reproduction of the Original on a reduced scale
Sheet 1

Fig. 1